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(54) Title: SEXING GENE

(57) Abstract

The present invention relates to the sequence of a sex-specific gene located on the avian female W chromosome (termed the ASW gene) and to the corresponding polypeptide. The present invention also relates to oligonucleotide probes which hybridise to the ASW gene and antibodies which bind to the ASW polypeptide. The present invention further relates to methods and kits for determining the sex of a bird.

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Sexing Gene

Field of invention

The present invention relates to a polynucleotide sequence which is located on the avian female specific W sex chromosome, and to polypeptides encoded by this sequence. The present invention also relates to methods of determining the sex of birds.

Background of invention

Commercial bird-breeding is a worldwide business enterprise and is particularly prevalent in the United States. Many commercially important birds, such as parrots, chickens and turkeys are difficult to sex using morphological characteristics. Behavioural observation is a commonly used method, but is time consuming and often inaccurate. A quick and accurate method of sexing birds would find a large market among commercial breeders.

Despite the commercial importance of numerous avian species, only limited studies have been conducted on the genetics and biochemistry of sex determination in birds. The sex chromosomes of birds differ from man and other mammals in that the female bird is the "heterogametic" sex having Z and W sex chromosomes. In mammals, the male is the "heterogametic" sex having both X and Y chromosomes whereas the female is "homogametic" having two X chromosomes.

The development of sex-specific genetic markers is desirable as such makers have the potential to provide valuable research tools useful for sex determination in birds. Such markers may be used, for example, to sexually identify immature birds prior to the development of gender specific morphological differences. Early sexual identification is an important consideration when breeding birds which become sexually mature prior to the development of external sexual characteristics. Genetic markers would also be useful in the breeding of rare bird species with unidentified secondary sexual characteristics.

Patent application No. PCT/US92/08284 (Zoogen Inc) describes one such genetic marker which is suitable for sex identification in avian species. The marker is a nucleic acid sequence which is present on both or one of the Z and W chromosomes of a number of bird species. In general, the nucleic acid sequence is used to produce probes which are capable of detecting restriction fragment length polymorphines (RFLPs) in DNA samples from

male and female birds. Disadvantages of this method are that the RFLP pattern from each sex can be difficult to determine and often requires testing of a known pair. Further, the genetic marker is not capable of determining the sex of all bird species. For example, the sex of penguins, raptors and Australian King Parakeets cannot be determined using this probe.

Disclosure of the invention

The present inventors have now identified and characterised a novel polynucleotide sequence which is specific to the avian female W chromosome. This sequence has been used to develop a genetic probe which allows rapid sex identification in almost all bird species.

Accordingly, in a first aspect the present invention provides an isolated polynucleotide, the polynucleotide having a sequence as set out in any one of Figures 1 to 5 or a sequence which hybridises thereto.

Also provided are a vector including such a polynucleotide, a host cell transformed with such a vector and recombinant proteins encoded by such a polynucleotide.

By "a sequence which hybridises thereto" we mean a sequence which hybridises under high or low stringency.

In a preferred embodiment, sequences derived from chickens which hybridise to the sequence shown in Figure 1 hybridise under high stringency. Sequences derived from other birds may hybridise under low stringency.

When used herein, "high stringency" refers to conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50^0C; (2) employ during hybridisation a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 nM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42^0C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS and 10% dextran sulfate at 42^0C in 0.2 x SSC and 0.1% SDS.

In a further preferred embodiment, the sequence which hybridises to the sequence shown in any one of Figures 1 to 5 shares at least 40%, more

preferably at least 60% and most preferably at least 80% homology with the sequence shown in Figure 1.

In a second aspect. the present invention provides an oligonucleotide probe of at least 8 nucleotides. the oligonucleotide probe having a sequence that hybridises to the polynucleotide of the first aspect of the present invention.

In a preferred embodiment the oligonucleotide probe does not hybridise under high stringency to the avian Z chromosome.

In a preferred embodiment the oligonucleotide probe is at least 10, more preferably at least 18 nucleotides.

It will be appreciated that the probes of the present invention may be produced by *in vitro* or *in vivo* synthesis. Methods of *in vitro* probe synthesis include organic chemical synthesis processes or enzymatically mediated synthesis. eg. by means of SP6 RNA polymerase and a plasmid containing the a ploynucleotide sequence according to the first aspect of the present invention under transcriptional control of an SP6 specific promoter.

In a further preferred embodiment the probe is conjugated with a label such as a radioisotope, an enzyme. biotin. a fluorescer or chemiluminescer.

The polynucleotide sequences and oligonucleotide probes of the present invention have a variety of uses in addition to their use in sexual identification. For example. the sequences may be used to screen recombinant DNA libraries prepared from a variety of avian species. The sequences may also be used in chromosome walking or jumping techniques to isolate coding and non-coding sequences proximal to the nucleotide sequence of the present invention. Further. sequences of the present invention may be used in the identification of sex determination mutations in avian species.

In a third aspect. the present invention provides an isolated polypeptide. the polypeptide encoded by the open reading frame as shown in Figure 2 or a biologically active fragment thereof.

By "biologically active fragment" we mean a fragment which retains at least one of the activities of the native polypeptide which activities include (i) the ability to induce production of antibodies which bind to the native polypeptide: and (ii) the ability to mimic the binding of the native polypeptide to at least one antibody or ligand molecule.

In a fourth aspect. the present invention provides an antibody which binds to a polypeptide according to the third aspect of the invention.

The term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus. the term covers antibody fragments. derivatives. functional equivalents and homologues of antibodies. including any polypeptide including an immunoglobulin binding domain. whether natural or synthetic. Chimaeric molecules including an immunoglobulin binding domain, or equivalent. fused to another polypeptide are therefore included.

In a preferred embodiment the antibody is conjugated to a label such as a radioisotope. an enzyme. biotin. a fluorescer or chemiluminescer.

In a fifth aspect. the present invention provides a method of determining the sex of a bird. which method includes analysing a biological sample derived from the bird for the presence of a polynucleotide according to the first aspect of the present invention.

It will be understood by a person skilled in this field that an analysis to determine whether a sample contains the polynucleotide sequence of the present invention may be performed in a number of ways. For example, the analysis may involve Southern hybridisation or dot blot hybridisation tests using probes according to the first or second aspects of the present invention.

Alternatively. the analysis may involve the technique of polymerase chain reaction (PCR). The term "polymerase chain reaction" or "PCR" when used herein generally refers to a procedure where minute amounts of a specific piece of nucleic acid. RNA and/or DNA. are amplified as described in U.S Patent No. 4,683.195, issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available. such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences. specific DNA sequences from total genomic DNA. and cDNA transcribed from total cellular RNA. bacteriophage or plasmid sequences. etc. See generally Mullis *et al.*. *Cold Spring Harbor Symp.* Quant. Biol. 51:263 (1987); Erlich. ed.. PCR Technology (Stockton Press. NY, 1989). As used herein. PCR is considered to be one. but not the only, example of a

nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of an established nucleic acid (DNA or RNA) as a primer, and utilises a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

In a sixth aspect, the present invention provides a method of determining the sex of a bird which method includes analysing a biological sample derived from the bird for the presence of a polypeptide according to the third aspect of the present invention.

It will be understood by a person skilled in this field that an analysis to determine whether a sample contains the polypeptide of the present invention may involve any suitable assay. For example, the polypeptide may be detected by an immunoassay involving an antibody according to the fourth aspect of the present invention. Suitable immunoassays include immuno-diffusion tests, immunoelectrophoresis, radioimmunoassays, affinity chromatography and Enzyme linked Immunoabsorbent Assays (ELISAs).

In preferred embodiments of the fifth and sixth aspects of the present invention, the bird is selected from Psittacines, Passerines, Penguins, Pigeons, Cranes rails and allies, chickens and kookaburras.

In a seventh aspect, the present invention provides a kit for sex determination of birds, which kit includes a polynucleotide according to the first aspect of the present invention, an oligonucleotide probe according to the second aspect of the present invention or an antibody according the fourth aspect of the present invention.

Advantages of the present invention reside in the fact that the novel sequence is specific to the avian W chromosome. This allows the development of simple and rapid determination tests based on detection of the polynucleotide sequence or corresponding polypeptide in biological samples obtained from birds. The present invention therefore allows the development of sex determination tests which do not require samples from birds of known sex. Further, as one suitable method for detecting the polynucleotide sequence involves PCR technology, it is envisaged that a single feather (or part thereof) would be sufficient material on which to perform a sex determination test. This would improve the speed and process of the test and would eliminate the current requirement for bird blood.

An additional advantage is that the present invention provides an avian sex determination test of wide application. The sex determination test described herein can be applied to most bird species including the following:-

- Psittacines - kakapo. crimson rosella
- Passerines - scrub wren. fairy wren
- Penguins - Adelie penguin. Fiordland crested penguin plus the related Black stilt
- Pigeons - New Zealand pigeon
- Cranes rails and allies - pukeko. takahe. Tasmanian hen. moorhen
- Chickens
- Kookaburras.

The present sex determination test is not applicable. however. to emus as they lack the heteromorphic sex chromosomes (ZW) which are found in other birds.

The terms "comprise". "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component or feature or group of components or features with or without the inclusion of a further component or feature or group of components or features.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described by way of the following non-limiting examples and Figures.

Brief description of the accompanying figures

Figure 1: Nucleotide and amino acid sequence corresponding to the ASW cDNA sequence.

Figure 2: cDNA sequence for ASW showing open reading frame (ORF).

Figure 3: Genomic sequence for ASW showing exon 1 and surrounding sequence.

Figure 4: Genomic sequence for ASW showing exon 2 and surrounding sequence.

Figures 5(a) and (b): Genomic sequence for ASW showing exon 3 and surrounding sequence.

Figure 6: Southern blot analysis of chicken DNA using the ASW probe. Panel A: high stringency conditions; Panel B: low stringency conditions.

Figure 7: Southern blot analysis of DNA derived from Kakapo (NZ parrot) using the ASW probe.

Figure 8: Southern blot analysis of Adelie Penguin DNA using the ASW probe.

Figure 9: Southern blot analysis of Fiordland Crested Penguin DNA using the ASW probe.

Examples

CLONING THE AVIAN SEX SPECIFIC GENE

A short 3' cDNA was identified using differential display as showing female specific expression in the chicken genital ridge. This transcript was cloned and originally termed 35B.

When used as a probe on a Southern blot of male and female chicken DNA the probe showed W linkage i.e bands corresponding to the probe were seen in females but not males. This has been confirmed on several independent chicken DNA samples. W linkage is also seen in numerous other bird species as outlined in the description. The gene has therefore been termed ASW (Avian sex-specific gene on the W).

The remaining 5' end of the cDNA was cloned using a 5' RACE system. The longest of these clones (identified in the laboratory as RACE G) when attached to the original clone 35B shows a continuous open reading frame (ORF) of 130 amino acids. The ORF contains limited homology to a putative protein kinase inhibitor from rat and cow.

A construct containing the complete cDNA of ASW has been prepared using the overlapping regions of 35B and RACE G. As above we have both plasmid DNA and glycerol stocks of this clone. The full cDNA sequence of the ASW clone is shown if Figure 1. Figure 2 shows the open reading frames in the cDNA sequence.

The cDNA clone was used as a probe to screen a female chicken genomic library. A genomic clone was isolated and relevant subfragments were subcloned and sequenced. Sequences of the genomic clones are shown in Figures 3 to 5.

All of the above clones are suitable for use on Southerns as a probe for sexing birds. All work quickly in other bird species indicating a significant degree of conservation across all birds.

SOUTHERN BLOT ANALYSIS

1. **Chicken Southern Blots**

A. **Electrophoresis and transfer of DNA**

Male and female chicken genomic DNA (10µg) was digested with *HindIII* overnight at 37°C. The digested DNA was electrophoresed on a large (20cm) 1% agarose gel at 15V overnight. The gel was then soaked in denaturing solution (1.5M NaCl: 0.5M NaOH) for 30 minutes, followed by two 15 minute washes in neutralising solution (1.5M NaCl: 0.5M Tris-HCl pH 7.2: 0.001M EDTA). DNA samples were then transferred to Hybond N^+ (Amersham) in 20X SSC (3M NaCl: 0.3M Na_3 citrate) overnight. DNA was then fixed to the membrane by soaking in 0.4M NaCl for 5 minutes.

B. **Preparation of ASW gene probe**

A plasmid containing the ASW clone was digested to release the insert. The digest was then electrophoresed on a 1% agarose gel and the insert run into low melt agarose. The ASW gene probe was then extracted from the agarose using agarase (Boehringer-Mannheim). The extracted insert was then roughly quantitated on an agarose gel.

ASW insert was labelled with ^{32}P-dCTP using random priming. The probe was then denatured at 100°C for 5 minutes. Renaturation of the probe was prevented by cooling on ice for 5 minutes.

C **Hybridisation of the ASW gene to genomic DNA**

Pre-hybridisation and hybridisation of Southern blots was performed in rotating bottles using a Hybrid oven. The Hybond N^+ membrane containing DNA was prehybridised for approximately two hours in hybridisation solution (5X Denhardts: 5X SSC: 0.1% SDS) at 65°C. Labelled probe was then added to the solution (2-3ng of probe per ml of hybridisation buffer) and hybridised overnight.

After hybridisation, washes were performed to remove non-specific binding of the probe. All washes were performed at 65°C.

If membranes were to be washed to high stringency, procedure was as follows:

Quick rinse with	5X SSC/0.1% SDS
Standing 2 minutes with	2X SSC/0.1% SDS
Rotating 10 minutes with	2X SSC/0.1% SDS
Rotating 30 minutes with	1X SSC/0.1% SDS
Rotating 30 minutes with	0.1X SSC/01.% SDS

If membranes were to be washed to low stringency, procedure was as follows:

Quick rinse with	2X SSC/0.1% SDS
Standing 10 minutes with	2X SSC/0.1% SDS
Rotating 15 minutes with	2X SSC/0.1% SDS

Sexing chickens is done at high stringency. Sexing of other birds requires low stringency washes.

D. **Autoradiography of Southern blots**

After washing, blots were exposed to film (Kodak X-OMat). High stringency chicken blot was exposed for 1 hour with two intensifying screens at -70^{0}C. Low stringency chicken blot was exposed for 3 hours with 2 intensifying screens at -70^{0}C. Low stringency blots of other birds were exposed for 4 days at -70^{0}C with 1 intensifying screen.

Results of Southern blot analysis using DNA samples derived from chicken, parrot and penguin species are depicted in Figures 6-9.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:-

1. An isolated polynucleotide, the polynucloetide having a sequence as set out in any one of Figures 1 to 5 or a sequence which hybridises thereto.
2. A vector which comprises a polynucleotide as claimed in claim 1.
3. A host cell comprising a vector as claimed in claim 2.
4. An oligonucleotide probe of at least 8 nucleotides in length, the oligonucleotide probe comprising a sequence which hybridises to the polynucleotide of claim 1.
5. An oligonucleotide probe according to claim 4 which does not hybridise under high stringency to the avian Z chromosome.
6. An oligonucleotide probe according to claim 4 or claim 5 which comprises at least 10 nucleotides.
7. An oligonucleotide probe according to claim 6 which comprises at least 18 nucleotides.
8. An oligonucleotide probe according to any one of claims 4 to7 wherein the probe is conjugated to a detectable label.
9. An oligonucleotide probe according to claim 8 wherein the label is selected from a radioisotope, an enzyme, biotin, a fluorescer or chemiluminiscer.
10. An isolated polypeptide, the polypeptide being encoded by the open reading frame as shown in Figure 2, or a biologically active fragment thereof.
11. An antibody which binds to a polypeptide according to claim 10.
12. An antibody according to claim 11 wherein the antibody is conjugated to a detectable label.
13. An antibody according to claim 12 wherein the label is selected from a radioisotope, an enzyme, biotin, a fluorescer or chemiluminiscer.
14. A method for determining the sex of a bird which comprises analysing a biological sample derived from the bird for the presence of a polynucleotide as claimed in claim 1.
15. A method according to claim 14 wherein the analysis involves probing the biological sample with an oligonucleotide as claimed in any one of claims 4 to 9.
16. A method according to claim 14 wherein the analysis involves performing a PCR on the biological sample using primers derived from the polynucleotide sequence as claimed in claim 1.

17. A method according to any one of claims 14 to 16 wherein the biological sample is derived from a feather of the bird.

18. A method for determining the sex of a bird which comprises analysing a biological sample derived from the bird for the presence of a polynucleotide as claimed in claim 10.

19. A method according to claim 18 wherein the analysis involves conducting an immunoassay using an antibody as claimed in any one of claims 11 to 13.

20. A method according to any one of claims 14 to 19 wherein the bird is selected from the group consisting of Psittacines. Passerines. Penguins. Pigeons. Cranes rails and allies. chickens and kookaburras.

21. A kit for determining the sex of a bird. the kit comprising a polynucleotide as claimed in claim 1.

22. A kit for determining the sex of a bird. the kit comprising an oligonucleotide probe as claimed in any one of claims 4 to 9.

23. A kit for determining the sex of a bird. the kit comprising an antibody as claimed in any one of claims 11 to 13.

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                9                  18                  27                  36                  45                  54
5' CCG AGC CGT GCT GAG CCG TGC TGA GCC GTG CTG GGG AGG TTT GGG GCT GAG GGA
   P   S   R   A   E   P   C   *   A   V   L   G   R   F   G   A   E   G

               63                  72                  81                  90                  99                 108
   GTG TTG TAG CGA GCG GGC GCC GTC ATG GCC GGC GGG ATC GTT AGG TCG CCG GCC
   V   L   *   R   A   G   A   V   M   A   G   G   I   V   R   S   P   A

              117                 126                 135                 144                 153                 162
   GCC TGG CGC GGT GGC GCC GCT CTC TTG GGA AAA GTC GCC CGC CAG GAG TTC TCC
   A   W   R   G   G   A   A   L   L   G   K   V   A   R   Q   E   F   S

              171                 180                 189                 198                 207                 216
   GCC AAC GTT ATC CGC GAG GAG GAG CCG TTG TGG ACG AGG AGT GCC TTG CGT TCC
   A   N   V   I   R   E   E   E   P   L   W   T   R   S   A   L   R   S

              225                 234                 243                 252                 261                 270
   ATG ATA TTT CAC CGC AAG CTC CTA CGC TTT TTC CTA GCC GCT CCC CAG AAG GCC
   M   I   F   H   R   K   L   L   R   F   F   L   A   A   P   Q   K   A

              279                 288                 297                 306                 315                 324
   GTT GTC GGG TTA TCT GGA GCA GAA GAT TGT GGC GCA CCT CTT CTT GGG CGT TTG
   V   V   G   L   S   G   A   E   D   C   G   A   P   L   L   G   R   L

              333                 342                 351                 360                 369                 378
   ATG ATT GTT GGC GAG AAG TGT GCT GCT AGC CTG GGC TTG ACC GAT GGA TTC CGG
   M   I   V   G   E   K   C   A   A   S   L   G   L   T   D   G   F   R

              387                 396                 405                 414                 423                 432
   ATG GCT GTG AGA TAC CCA CCC TCA GTC CCT TCA GAC TAC CGC GCG CGG CTC TGT
   M   A   V   R   Y   P   P   S   V   P   S   D   Y   R   A   R   L   C

              441                 450                 459                 468                 477                 486
   ATT CTG GGT GGC CGT CAG TTG GGC CAG CCT CCT GGC TAA GAT GTT TGC ACC GCC
   I   L   G   G   R   Q   L   G   Q   P   P   G   *   D   V   C   T   A

              495                 504                 513                 522                 531                 540
   GGT GTT GCT GCA CGC GTA CGG ATC GCC ACC GAA TGG GTT TCA CGT GTT GCC CGT
   G   V   A   A   R   V   R   I   A   T   E   W   V   S   R   V   A   R

              549                 558                 567                 576                 585                 594
   CAG CCT AGC CAC CGG TGA CAT GTA ATT GTT TTT GGT GGG TGA CTA TGG AGG GTA
   Q   P   S   H   R   *   H   V   I   V   F   G   G   *   L   W   R   V

              603                 612                 621                 630                 639                 648
   ATG AAA AGC TTT GAG CAG CAT TTG CAG AAT AAA GAT GGA GCA TGG GGA TAT CAA
   M   K   S   F   E   Q   H   L   Q   N   K   D   G   A   W   G   Y   Q

              657
   AAA AAA AAA 3'
   K   K   K
```

Figure 1

cDNA SEQUENCE FOR ASW SHOWING OPEN READING FRAME (ORF)

CCGAGCCGTGCTGAGCCGTGCTGAGCCGTGCTGGGGAGGTTTG

GGGCTGAGGGAGTGTTGTAGCGAGCGGGCGCCGTCATGGCCGG ↓Start of ORF

CGGGATCGTTAGGTCGCCGGCCGCCTGGCGCGGTGGCGCCGCT

CTCTTGGGAAAAGTCGCCCGCCAGGAGTTCTCCGCCAACGTTA

TCCGCGAGGAGGAGCCGTTGTGGACGAGGAGTGCCTTGCGTTC

CATGATATTTCACCGCAAGCTCCTACGCTTTTTCCTAGCCGCT

CCCCAGAAGGCCGTTGTCGGGTTATCTGGAGCAGAAGATTGTG

GCGCACCTCTTCTTGGGCGTTTGATGATTGTTGGCGAGAAGTG

TGCTGCTAGCCTGGGCTTGACCGATGGATTCCGGATGGCTGTG

AGATACCCACCCTCAGTCCCTTCAGACTACCGCGCGCGGCTCT

GTATTCTGGGTGGCCGTCAGTTGGGCCAGCCTCCTGGCTAAGA ↓end of ORF

TGTTTGCACCGCCGGTGTTGCTGCACGCGTACGGATCGCCACC

GAATGGGTTTCACGTGTTGCCCGTCAGCCTAGCCACCGGTGAC

ATGTAATTGTTTTTGGTGGGTGACTATGGAGGGTAATGAAAAG

CTTTGAGCAGCATTTGCAGAATAAAGATGGAGCATGGGGATAT

CAAAAAAAAAAA

Figure 2

GENOMIC SEQUENCE FOR ASW SHOWING EXON 1 AND SURROUNDING SEQUENCE

CGGCGCTCTGTCGGCCCAATGAGCGCCGCCGAGGGCGGGACCG

↓Start of exon 1

GGCCGAGGCGAGCCGAGCCGTGCTGAGCCGTGCTGAGCCGTGC

TGGGGAGGTTTGGGGCTGAGGGAGTGTTGTAGCGAGCGGGCGC

CGTCATGGCCGGCGGGATCGTTAGGTCGCCGGCCGCCTGGCGC

GGTGGCGCCGCTCTCTTGGGAAAAGTCGCCCGCCAGGAGTTCT

End of exon 1 ↓

CCGCCAACGTTATCCGCGAGGAGGAGCCGTTGTGGACGAGGAG

GTAGTTG

Figure 3

GENOMIC SEQUENCE FOR ASW SHOWING EXON 2 AND SURROUNDING SEQUENCE

GGTGTTCTTGAGGATGGAGAGTTAACACGGCCTCCGTCGGGAT

AACCCCAGGTTCTTTGAAAATGCCTTGGTCAAAAGTAGGATAG

GAAGGCGATATTTGGCCATAACTGAAGGATGGTGAGCCACCGT

TTGCCGCTTGAGTGGTTAAGCAGGGTCTTAATATGAGAGTGAA

ATAAGCGCAAATGGAGGTGCTTTTGTTTGGGTTTAAAATCGCT

CTGTGCTCGTAGCAGCAGGAGCCTGTGAAAACATGTCTTGTGC

ATAGAAGGGAGAGGGCTTTGCCGATTCAAAGATCCTTAGGAAG

AATCGCTGTTTGTCTGCGTTGGGACGCGTTCAGTGGGGCGTAG

CGATCTGCTTCAGCTATTGCCTTTCTCCGAAGCCAATCCCGTT

↓ Start of exon 2

TTAAGTGTGTCCTCTTCCTCCAGTGCCTTGCGTTCCATGATAT

TTCACCGCAAGCTCCTACGCTTTTTCCTAGCCGCTCCCCAGAA

End of exon 2 ↓

GGCCGTTGTCGGGTTATCCGGAGCAGAAGATTGTGGCGCACCT

GTAAGTACCGTGGAAGCTTTCTGTACACGAAACGGTGCCAGTA

AGTGGTATGTAATTTAGTAGGCTCTGTCCCGTCGCCTCGTTTC

CTCTCGATGTTGCAAAGAGGCACAGTAGGCTATTTGGGCTTTC

CGACGCATAATC

Figure 4

GENOMIC SEQUENCE FOR ASW SHOWING EXON 3 AND SURROUNDING SEQUENCE

CTTGGGTTTTGAGCAGGTCGGAGGCGAGGAAGGGGAAAGCTGG

CTGAGGGGTGCTGGATGTCTCATTTTCAGCTGTAGAAGTCAAG

TCCCGTGGCTTTGGCGTGGGCTCACAAAGCCTACACTGAGTTT

TTCTTTTCTGCCGGAAAGACTTAGGTTGCCCGAAGATATAATG

GGGGGCTGGAAGTCCGAGACAAATCTGGCTTCTGTGCCCGATT

TGTAGTCTTGACTGTCTAGCTGGGGGTGGGGAGGGGGTTAAAA

AAAAAAAAGCAAAAAAAAGGTGGCAAGTCCCATAGCTGCCCCT

CCCCCCATTATGCATAATCGGTCTTTGTAGCTTTGCTTGGAAT

GTGTCAGTTCGCAGAAAGGCAGTAATTTGAAGAGCGGCCCTTG

AATGGCGAAGAACTAGTTAACCGCGTTCTGTGGCTGGTTCTCT

GCTCGCCTTCCTTGGCTTCCTGCCCTCTCCACCCTTCATTGTG

Start of exon 3 ↓

TTGTGGCATGCTCCGTTACGTAACGTTATTTCCTTCTCCAGCT

TCTTGGGCGTTTGATGATTGTTGGCGAGAAGTGTGCTGCTAGC

CTGGGCTTGACCGATGGATTCCGGATGGCTGTGAGATACCCAC

CCTCAGTCCCTTCAGACTACCGCGCGCGGCTCTGTATTCTGGG

TGGCCGTCAGTTGGGCCAGCCTCCTGGCTAAGATGTTTGCACC

GCCGGTGTTGCTGCACGCGTACGGATCGCCACCGAATGGGTTT

CACGTGTTGCCCGTCAGCCTAGCCACCGGTGACATGTAATTGT

Figure 5(a)

TTTTGGTGGGTGCCTATGGAGGGTAATGAAAAGCTTTGAGCAG

End of exon 3 ↓

CATTTGCAGAATAAAGATGGAGCATGGGGATATCATCTCTGTC

TCGCGTGTCTTACTGATGGGAATGGTTCTGCAAGTTAAAACGG

TGGGTCTCGTCTCCAAAGATAATATAGGATGTGCTGTGTGTTT

GAGGCCTTGCTGTCTTCGCAGCTCAATAAAGGCGTGGTTTATC

GCGTACTAGCAGCATCGACAAGCAGCAGCGTAGAATTAGCGAA

GAATAAGGTAGGAAGAAAGCTTCCAGCTGTCGTCTGTTGTATG

GCCCCGTCGCGAGGCCAAATAAACTCTACCTGCATGATTTCTG

ACCGGTGCGCGTCAGCCTGTTCTTAACCAAACTCGGAGTTTTG

CCACTGCTTTCTTTTCAGTCGCGGATTTTGCGACGTAGAGAAT

TAATTTCTCCC

Figure 5(b)

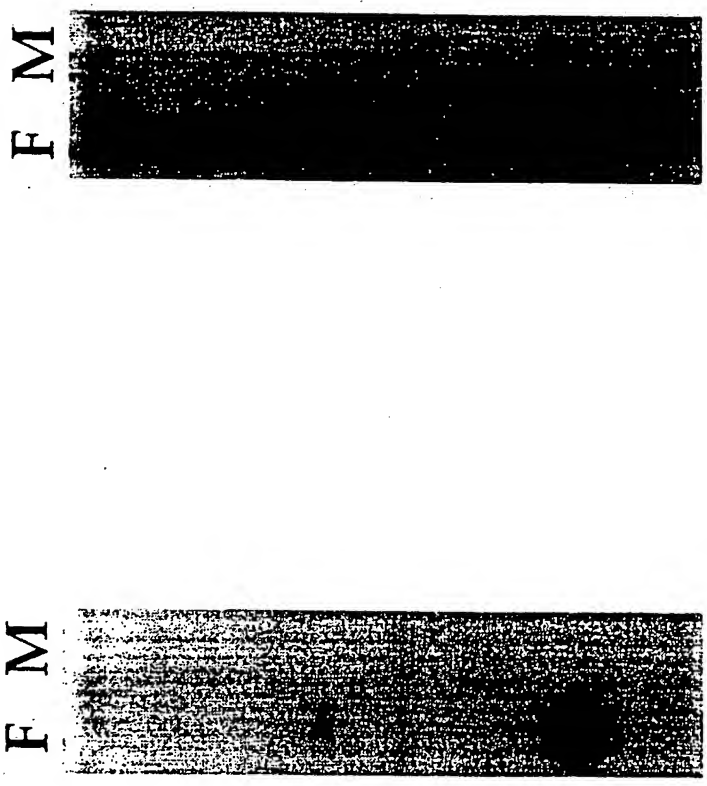

Figure 6

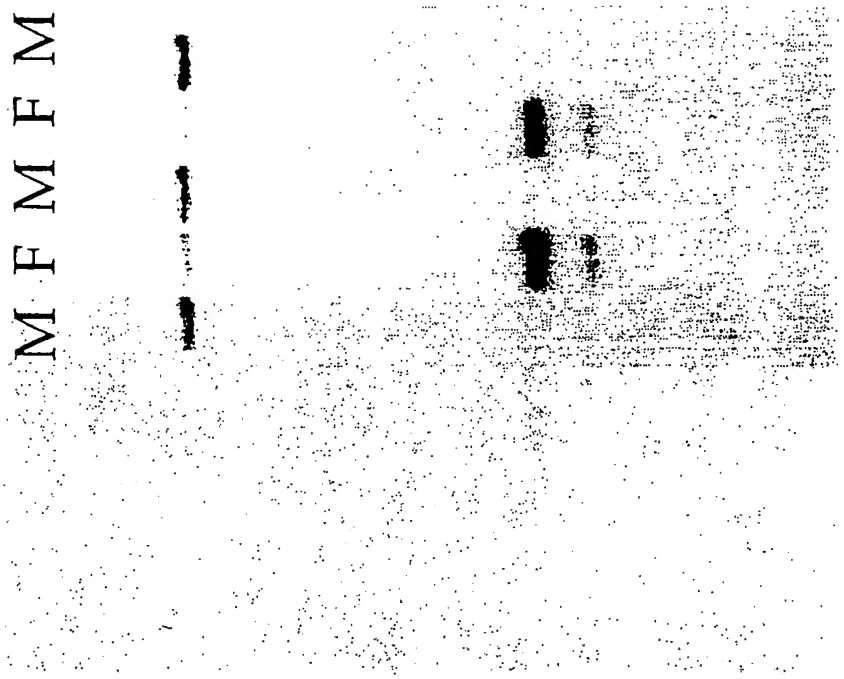

Figure 7

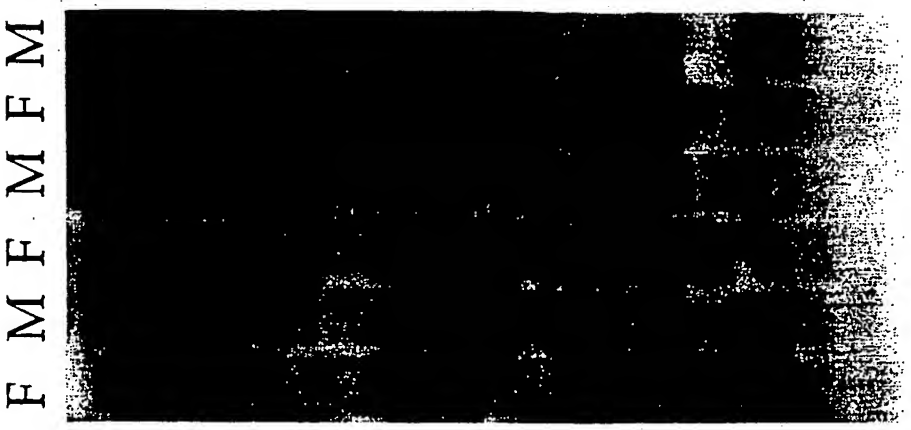

Figure 9

Figure 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00396

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N - 15/12, C07K - 14/465 16/18 C12Q-1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
CHEMICAL ABSTRACTS: See below
WPAT : BINDER/IN OR SINCLAIR/IN

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DNA Sequence of figures 1-4 searched : Swiss Prot, Genbank, EMBL, PIR
Cas online, STN (DGENE) : figure 1 nucleotides 79-272 and 273-332

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Neurochemical Research 19, pages 575-580 (1994) Kurosawa, N. et al "Molecular Cloning and Characterization of Avian N-Methyl-D-Aspartate Receptor Type 1 (NMDA-R1) Gene" see figure 2	1-9
X	J. Biol. Chem 269, pages 212-219 (1994) Nimpf, J et al "The somatic Cell-Specific low density lipoprotein receptor-related protein of the chicken" see figure 2	1-9

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search
18 August 1997

Date of mailing of the international search report
22 AUG 1997

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00396

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Mol. Evol. 36 pages 255-262 (1993) shartzer, K.L. et al "Evolution of Avian metallothionein: DNA sequence analyses of the turkey metallothionein gene and metallothienein cDNAs from pheasant and quail see figure 2	1-9